# Potential of *Agrotis ipsilon* Nucleopolyhedrovirus for Suppression of the Black Cutworm (Lepidoptera: Noctuidae) and Effect of an Optical Brightener on Virus Efficacy

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**ABSTRACT** Studies were performed in the laboratory, greenhouse and field to assess the potential of *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (AgipMNPV) and a viral enhancing agent, M2R, for suppression of *Agrotis ipsilon* (Hufnagel). In laboratory droplet feeding bioassays, AgipMNPV was shown to be highly active against third-instar *A. ipsilon*. The optical brightener M2R significantly reduced $LD_{50}$ estimates by ≈160-fold, but had no direct effect on survival time estimates. In greenhouse trials, spray and bait formulations of AgipMNPV significantly reduced feeding damage to corn seedlings caused by third-instar *A. ipsilon*. In two sets of replicated field trials, bait formulations of AgipMNPV significantly reduced feeding damage to corn seedlings by third-instar *A. ipsilon*. However, there were no beneficial effects attributable to the inclusion of M2R in AgipMNPV formulations under greenhouse or field conditions. It seems likely that in an appropriately designed pest management program AgipMNPV could be used to suppress field populations of early and mid-instar *A. ipsilon*.

**KEY WORDS** *Agrotis ipsilon*, baculovirus, optical brightener, M2R

THE BLACK CUTWORM, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is a worldwide pest of over 30 important crops (Rings et al. 1975), and in the U.S. Corn Belt, *A. ipsilon* can be a serious localized pest of field corn (Clement and McCartney 1982, Engelken et al. 1990). All instars of *A. ipsilon* feed on the leaves of corn seedlings, but the most serious damage results from leaf and stem cutting by late instars (Clement and McCartney 1982). Present management of *A. ipsilon* is based on population monitoring and rescue applications of chemical insecticide against damaging larval populations (Stockdale 1977).

A baculovirus, *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (AgipMNPV) with potential for use in managing *A. ipsilon* was recently characterized (Boughton et al. 1999). However, to date, baculoviruses have not been widely used as microbial insecticides for the management of insect pests, primarily due to the following four main factors limiting their use (Moscardi 1999): (1) high production costs, (2) low biological activity (slow to kill), (3) low activity against late instars, and (4) low persistence due to degradation by ultraviolet (UV) light. A range of compounds have been evaluated as potential adjuvants for inclusion in baculovirus formulations to overcome some of these inherent biological limitations. Among these compounds a group of stilbene disulfonic acid derivatives known as optical or fluorescent brighteners have shown particular promise (Shapiro 1992). Optical brighteners absorb radiation in the nonvisible UV part of the electromagnetic spectrum and then reemit this radiation as visible light in the blue part of the spectrum. These compounds have long been used in the textile and detergent industries where their fluorescence activity makes clothes appear whiter and brighter.

Shapiro initially investigated optical brighteners as possible UV protectants for inclusion in formulations of the gypsy moth nucleopolyhedrovirus (LdMNPV) (Shapiro 1992). Subsequently it was found that not only did these compounds offer protection against UV light, but they also enhanced virus-induced mortality in gypsy moth, *Lymantria dispar* (L.), larvae (Shapiro and Robertson 1992). When second-instar *L. dispar* were fed artificial diet laced with LdMNPV and the optical brightener M2R (Calcofluor white M2R, Tinopal LPW, fluorescent brightener 28), lethal concentrations were reduced 529–1670 fold relative to the virus alone, and the time taken for 50% of exposed larvae to die from virus infection ($LT_{50}$) was lowered from 11.2 d for the virus alone to 6.0 d for the virus and brightener (Shapiro and Robertson 1992). In addition, optical brighteners were also found to greatly enhance the activity of LdMNPV against later instar gypsy moth larvae (Webb et al. 1994a). Since then, studies in a range of baculovirus-host systems have shown optical brighteners to function as enhancers of viral activity (Hamm and Shapiro 1992, Farrar et al. 1995, Vail et al.

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1996, Zou and Young 1996, Argauer and Shapiro 1997, Li and Otvos 1999). Several studies have also shown that optical brighteners can act as enhancers of baculovirus activity under field conditions, either by elevating pest mortality relative to treatment with virus alone (Hamm et al. 1994, Li ans Otvos 1999, Vail et al. 1999) or by allowing similar levels of mortality to be achieved using lower application rates of virus (Webb et al. 1994a, Thorpe et al. 1998).

We report the results of laboratory bioassays to evaluate enhancement of AgipMNPV activity by the optical brightener M2R, against *A. ipsilon* larvae. We also present the results of greenhouse and field trials to evaluate the potential of AgipMNPV and M2R for suppression of *A. ipsilon.*

## Materials and Methods

**Insects.** *Agrotis ipsilon* eggs were obtained from a colony maintained by USDA-ARS Corn Insect and Crop Genetics Research Unit (Ames, IA). Eggs were placed in an incubator at 28°C with a photoperiod of 12:12 (L:D) h. After emergence, groups of 50 newly emerged first instars were transferred to 237-ml ice cream cups containing artificial pinto bean diet (Hendrix et al. 1991). Larvae were reared to the third instar, and were either used at this developmental stage, or were transferred individually into 17-ml jelly cups (Fill-Rite, Newark, NJ) containing diet, and maintained at 28°C until they reached the desired developmental stage.

**Virus.** AgipMNPV polyhedral occlusion bodies (OBs) were quantified with a phase contrast microscope and a Neubauer bright-line hemocytometer (Fisher, Pittsburg, PA). OBs used in the lethal dose and lethal time bioassays originated from the same virus stock used for the initial host range screening studies (Boughton et al. 1999). This stock was produced by a single amplification passage through *A. ipsilon* larvae and was purified by a series of centrifugation and washing steps. The OBs used in the greenhouse and field studies were produced by a second amplification passage through *A. ipsilon* larvae as described below. Three hundred fourth-instar *A. ipsilon* were starved overnight and inoculated orally with suspensions of OBs, using a modified droplet feeding technique (Hughes and Wood 1981). Using a 10 $\mu$l micropipette (Rainin Instrument, Woburn, MA), 2 $\mu$l of a 500,000 OB per microliter suspension of AgipMNPV, containing 4% blue food coloring dye, was pipetted directly in front of the starved larva in each jelly cup, and the larva allowed to drink. Larvae that fully ingested the droplet of virus suspension were provided with artificial diet, and maintained at 28°C. Larvae that failed to ingest the entire droplet were discarded. Larvae were monitored daily from 4 d after inoculation, and following death, cadavers were frozen and polyhedra extracted as described previously (Boughton et al. 1999). Sodium azide was added to polyhedral preparations to a final concentration of 0.02% to prevent bacterial growth, and virus stocks were stored at 4°C.

**Lethal Dose Bioassays.** Third-instar *A. ipsilon* housed individually in 17-ml jelly cups were starved overnight and inoculated with suspensions of AgipMNPV in water or AgipMNPV in 0.5% (W:V) M2R. For bioassays of OBs in water, concentrations of 0, 250, 750, 1,000, 1,500 and 3,000 OBs/$\mu$l were used, while for bioassays of OBs in 0.5% M2R, concentrations of 0, 1, 3, 9, 27 and 81 OBs/$\mu$l were used. All suspensions contained 4% blue food coloring dye and larvae were inoculated with a 1-$\mu$l droplet of virus suspension, using the modified droplet feeding technique described above. Larvae that failed to ingest the entire droplet of fluid were discarded. Sufficient larvae were inoculated at each dose to obtain 35 larvae that had ingested the entire droplet. After inoculation, cubes of diet were introduced into each jelly cup and the larvae were subsequently maintained at 28°C. Bioassays were replicated three times. Mortality was scored 9 d after infection. Data were subjected to probit analysis (Russell et al. 1977) and the assumptions of the models verified (Robertson and Preisler 1992b).

**Survival Time Bioassays.** The bioassays used to evaluate the mortality-time relationship between AgipMNPV and *A. ipsilon* were such that larvae were exposed to brightener and virus for only a short period of time ($\approx$40 min) before being transferred to fresh diet, as opposed to chronic exposure in diet-surface contamination bioassays, in which larvae are exposed to the virus and brightener for the duration of the study. As such our bioassays estimated median survival time (ST) rather than median lethal time (Farrar and Ridgway 1998). Third-instar *A. ipsilon* in separate 17-ml jelly cups were starved overnight and inoculated with suspensions of AgipMNPV in water with or without 0.5% M2R using the droplet feeding technique described above. In each survival time bioassay, larvae were inoculated with OBs in water and OBs in 0.5% M2R at $LD_{50}$ and $LD_{99}$ doses. For OBs in water, the $LD_{50}$ and $LD_{99}$ consisted of 1-$\mu$l droplets of 433 OBs/$\mu$l and 11,918 OBs/$\mu$l, respectively. For OBs in 0.5% M2R, the $LD_{50}$ and $LD_{99}$ consisted of 1-$\mu$l droplets of suspensions of 3 OBs/$\mu$l and 27 OBs/$\mu$l respectively. Larvae that failed to ingest the entire 1-$\mu$l droplet were discarded. Sufficient larvae were inoculated with both virus treatments to obtain 40 and 80 larvae at the $LD_{99}$ and $LD_{50}$, respectively, so that when survivors were excluded from the results, survival time analyses would be based on similar numbers of datapoints. In each bioassay, a batch of larvae was first inoculated with the $LD_{50}$ of OBs in water, then 1 h later another batch of larvae was inoculated with the $LD_{50}$ of OBs in 0.5% M2R. At 1-h intervals subsequently, batches of larvae were inoculated with the $LD_{99}$ of OBs in water and finally the $LD_{99}$ of OBs in 0.5% M2R. Controls consisting of 20 larvae inoculated with water and 4% food dye with or without 0.5% M2R were set up with each bioassay to monitor for nonviral mortality. After inoculation, cubes of diet were introduced into each jelly cup and the larvae maintained at 28°C. Mortality was recorded 55 h after inoculation and every 4–6 h until there was no further mortality. Bioassays were replicated twice. Data were analyzed

using the Kaplan-Meier product-limit estimator method (Kabfleisch and Prentice 1980). Median $ST_{50}$ estimates were compared using log-rank tests (Kabfleisch and Prentice 1980).

**Greenhouse Studies.** Greenhouse studies were carried out to assess the potential of AgipMNPV and M2R for reducing feeding damage to corn seedlings caused by *A. ipsilon* larvae. Eight treatments were used in the greenhouse studies. Four treatments were formulated on a wheatgerm bait, while the other four treatments were applied as aqueous solutions. The four bait treatments consisted of wheatgerm bait that had been sprayed with distilled water, a solution of 0.5% M2R, AgipMNPV in distilled water, or AgipMNPV in 0.5% M2R. Solutions were sprayed on to the bait at a rate of 0.5 ml/g of bait, using two plant misters, one of which was used exclusively for virus suspensions and another of which was used exclusively for nonvirus solutions. Virus concentrations were adjusted to obtain rates of $3.13 \times 10^8$ OBs/g of wheatgerm bait. Baits were allowed to dry and stored at room temperature. The four treatments applied as aqueous solutions consisted of (1) distilled water, (2) a solution of 0.5% M2R, (3) AgipMNPV in distilled water, and (4) AgipMNPV in 0.5% M2R.

Trays (36 by 51 by 10 cm) filled to a depth of 8 cm with sieved soil were planted with Pioneer 3489 corn in two rows, with five seeds per row. The seeds were planted at 5-cm intervals with 15 cm between the rows. Strips of aluminum lawn edging 16 by 180 cm were pushed into the soil around the edges of each tray, to form a continuous barrier, and the tops of these barriers were smeared with Vaseline to prevent larval escape. The trays were watered daily and maintained in a greenhouse with a maximum temperature of 26°C and minimum of 18°C and a photoperiod of 16:8 (L:D) h. Treatments were randomly assigned to trays and were applied when seedlings reached the two-leaf stage. Baits were sprinkled on to the surface of the soil in each tray at a rate of 2.24 g/m$^2$ which gave a virus application rate of $7 \times 10^8$ OBs/m$^2$. Ten ml of each aqueous solution was sprayed onto each soil flat, and concentrations of AgipMNPV were adjusted to obtain application rates of $7 \times 10^8$ OBs/m$^2$. Trays that were to be treated with aqueous solutions were removed from the greenhouse during the application process to avoid spray-drift contamination of other trays. Once all the trays had been treated, 15 third-instar *A. ipsilon* were released into the center of each tray. Trays were subsequently watered daily and feeding damage was scored 7 d after treatment. The 10 seedlings in each tray were each assigned a damage rating on a five-point scale, 1 for undamaged seedlings, 2 for seedlings with shot-holes in the leaves, 3 for seedlings with chewed leaf edges, 4 for seedlings with leaves chewed off, and 5 for seedlings with stems cut through. These 10 damage ratings were then averaged to obtain a mean damage rating for that treatment. The greenhouse trials were replicated four times using fresh baits, soil and trays for each trial. All bait preparations were bioassayed with third-instar *A. ipsilon* to assess mortality. Larvae were placed in separate jelly cups and allowed to feed overnight on an excess (≈0.03 g) of each wheatgerm bait. Twenty-five larvae were used for each bait treatment and after feeding, larvae were moved into clean jelly cups containing cubes of fresh diet. Larvae were maintained at 28°C and mortality scored after 8 d.

Mean feeding damage ratings for each treatment from the four replicates were $\log_{10}$ transformed and analyzed by one-way analysis of variance (ANOVA) using Tukey's test to separate means, and subsequently by factorial ANOVA using the General Linear model (MINITAB 1991). Data sets satisfied the assumptions of normality of error and homogeneity of variance.

**Field Trials.** Field trials were carried out during May 2000 to assess the effectiveness of bait formulations of AgipMNPV with or without M2R, at reducing feeding damage to corn seedlings caused by *A. ipsilon* larvae. Trials were conducted at an Iowa State University research site, Johnson Farm, Ames, IA. A field was machine planted with Garst 8543 corn, using a row spacing of 76 cm, on 2 May 2000. After emergence of the corn, barriers consisting of lawn edging (610 by 13 and 381 by 13 cm) (Suncast, Batavia, IL) were dug into the ground to a depth of 5 cm around five adjacent rows of corn, to form blocks 610 by 381 cm in size. Four additional barriers (610 by 13 cm) were then dug into the ground within each block, between the rows of corn seedlings. Each block thus contained five treatment plots, and each plot consisted of a row of ≈30 corn seedlings, fully enclosed by barriers, to prevent larval escape. The trials were set up according to a randomized complete block design, with four blocks. The greenhouse trials revealed no differences in the effectiveness of spray and bait formulations of AgipMNPV, but because of their ease of handling, bait treatments were selected for use in the field studies. Treatments consisted of the same four baits used in the greenhouse studies plus an untreated control, and were randomly assigned to plots when seedlings reached the two-leaf stage. The baits were produced as described for the greenhouse studies, except that wheatbran was used in place of wheatgerm. Wheatbran proved to be less sticky when damp than wheatgerm, and consequently wheatbran baits were easier to produce and gave better coverage in the field than wheatgerm. Baits were produced immediately before application and were applied to plots just before sunset at the same application rates as were used in the greenhouse studies. After application of treatments, plots were infested with third-instar *A. ipsilon* by sprinkling larvae along the center line of each row at a rate of three larvae per seedling. Feeding damage was scored 7 d after application of treatments using the same five-point damage rating scale used in the greenhouse studies. Damage ratings for each of the seedlings in a row were averaged to obtain a mean damage rating for that treatment. This was done for each treatment in each of the four blocks. All bait preparations were bioassayed with third-instar *A. ipsilon* as described for the greenhouse studies. The trials were repeated a second time in a different area of the field

**Table 1. Pooled lethal dose data for AgipMNPV in third-instar *A. ipsilon***

| Treatment[a] | $n$[b] | $LD_{50}$[c] (95% CL) | Slope ± SE |
|---|---|---|---|
| Agip | 630 | 330 (83–548) | 1.57 ± 0.18 |
| Agip M2R | 630 | 2.1 (1.8–2.5) | 2.28 ± 0.20 |

[a] Agip, AgipMNPV suspended in water; Agip M2R, AgipMNPV suspended in 0.5% M2R.

[b] For both treatments, data from three replicates indicated equivalent dose-mortality responses, by likelihood ratio test of equality (Robertson and Preisler 1992a), so data were pooled. Six virus doses per replicate, with 35 larvae per dose.

[c] Dose, OBs per larva. Data analyzed by probit analysis (Russell et al. 1977). Probit-dose relationship linear at $P = 0.05$ confidence level. Data fit probit model by chi-square test at $P = 0.05$. Comparison of treatments by chi-square tests on slopes and intercepts indicates that the $LD_{50}$ for AgipMNPV in 0.5% M2R is significantly lower and that the slope is significantly greater than for AgipMNPV in water at the $P = 0.05$ significance level (Robertson and Preisler 1992a).

**Table 2. Survival time data for AgipMNPV in third-instar *A. ipsilon***

| Treatment[a] | $ST_{50}$[b] | | | |
|---|---|---|---|---|
| | $LD_{50}$[c] | $LD_{99}$ | $LD_{50}$[c] | $LD_{99}$ |
| | Replicate 1 | | Replicate 2 | |
| Agip | 123aA | 101aB | 127aB | 121aB |
| Agip M2R | 127aA | 108aB | 137aA | 121aB |

[a] Agip, AgipMNPV suspended in water; Agip M2R, AgipMNPV suspended in 0.5% M2R.

[b] Survival time, hours. Estimates obtained using the Kaplan-Meier product-limit estimator method (Kabfleisch and Prentice 1980).

[c] Replicates used 80 larvae at the $LD_{50}$ level and 40 larvae at the $LD_{99}$. Survivors were excluded from analyses. Within replicates, $ST_{50}$ estimates for different doses and treatments were compared by log-rank tests (Kabfleisch and Prentice 1980). $ST_{50}$ estimates in the same column, followed by the same lower-case letter do not differ significantly at $P = 0.05$ level. $ST_{50}$ estimates in the same row, followed by different upper-case letters differ significantly at $P = 0.05$ level.

on corn that was planted on 16 May 2000. A second batch of baits was prepared, but all other aspects of the experimental design were the same as for the first set of field trials.

For both field trials, mean feeding damage ratings for each treatment from the four blocks were $\log_{10}$-transformed and analyzed by factorial ANOVA using the General Linear model (MINITAB 1991). Data sets satisfied the assumptions of normality of error and homogeneity of variance.

## Results

**Lethal Dose Bioassays.** AgipMNPV proved to be highly active against third-instar *A. ipsilon.* For the AgipMNPV in water and AgipMNPV in 0.5% M2R treatments, data from the three replicates showed equivalent dose-mortality responses by likelihood ratio tests of equality (Robertson and Preisler 1992a), so the data were pooled. The $LD_{50}$ estimate for AgipMNPV in water was 330 OBs per larva, while for AgipMNPV in 0.5% M2R the $LD_{50}$ estimate was significantly lower at 2.1 OBs per larva (Table 1). For AgipMNPV in M2R, the intercept of the dose–response curve was significantly lower and the slope significantly higher than for AgipMNPV in water. This indicates that *A. ipsilon* is significantly more susceptible to infection, and that significantly greater mortality occurs per unit increase in virus dose when AgipMNPV is suspended in M2R, than when AgipMNPV is suspended in water (Robertson and Preisler 1992a).

**Survival Time Bioassays.** $ST_{50}$ estimates for both virus treatments were between 4 and 5 d in both bioassay replicates (Table 2). Comparisons made at the $LD_{50}$ dose level, showed that there were no significant differences between $ST_{50}$ estimates for AgipMNPV and AgipMNPV in 0.5% M2R. There were also no significant differences at the $LD_{99}$ level between $ST_{50}$ estimates for AgipMNPV and those for AgipMNPV in 0.5% M2R. As expected for both AgipMNPV and AgipMNPV in 0.5% M2R, $ST_{50}$ estimates were significantly lower at the $LD_{99}$ level than at the $LD_{50}$ level ($P < 0.05$).

**Greenhouse Studies.** One-way ANOVA showed that treatment explained a significant amount of the variation seen in feeding damage in the greenhouse ($F = 21.09$; df = 7, 24; $P = 0.0001$). Mean damage ratings for the four treatments containing AgipMNPV were not significantly different from each other, nor were the mean damage ratings for the four non-AgipMNPV treatments (Fig. 1). Trays treated with for-

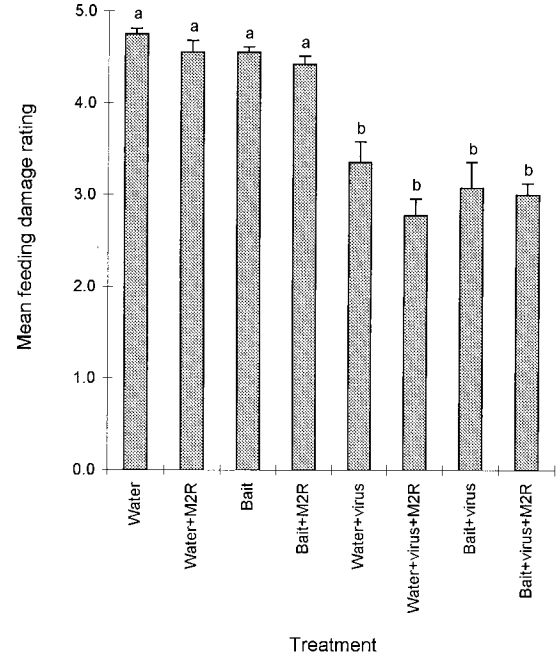


**Fig. 1.** Mean feeding damage ratings averaged across four replicates, for the eight treatments used in the greenhouse studies. Damage rating of five corresponds to cut seedlings while one corresponds to undamaged seedlings (see *Materials and Methods*). Bars show standard errors. Columns with the same letter do not differ at $P = 0.05$ significance level by Tukey's means separation test. Columns with different letters are different at $P = 0.05$ significance level.

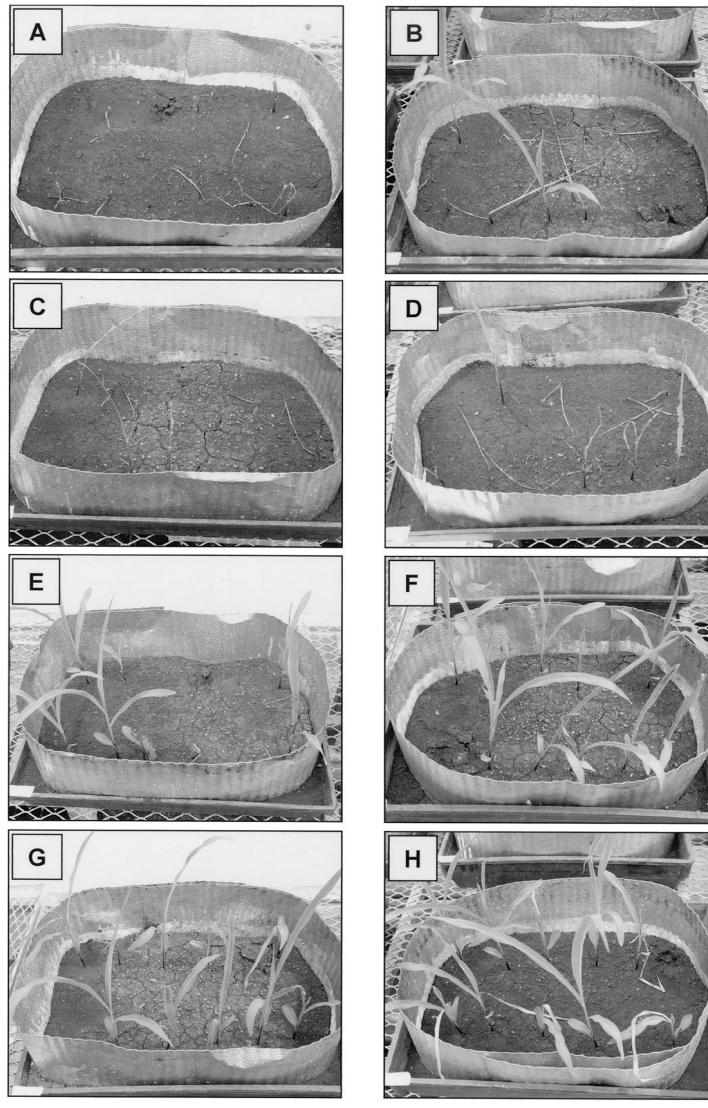


**Fig. 2.** Trays of corn seedlings from the first replicate of the greenhouse studies, showing typical feeding damage observed after 7 d for trays receiving different treatments. Treatments were as follows: (A) water only, (B) 0.5% M2R, (C) bait only, (D) bait + 0.5% M2R, (E) AgipMNPV in water, (F) AgipMNPV in 0.5% M2R, (G) bait + AgipMNPV, (H) bait + AgipMNPV in 0.5% M2R.

mulations containing AgipMNPV exhibited significantly lower feeding damage ratings than did trays receiving nonvirus treatments. Typical levels of feeding damage observed in trays receiving different treatments are shown in Fig. 2. Photographs (A), (B), (C), and (D) show examples of feeding damage observed in trays that received nonvirus treatments. Photographs (E), (F), (G), and (H) show the lower levels of feeding damage typical of trays that received treatments containing virus.

In the initial factorial ANOVA, all possible interactions between virus, brightener and formulation were included in the model, but none of these terms proved to be significant. In a subsequent analysis that excluded interaction terms, virus was the most significant factor explaining differences in observed feeding damage, although replicate and brightener also accounted for significant amounts of variation in feeding damage (Table 3). Laboratory bioassays of baits from the greenhouse trials resulted in 100% mortality of larvae fed baits containing AgipMNPV but no mortality in larvae fed non-AgipMNPV baits.

**Field Trials.** Mean feeding damage ratings for each of the five treatments were averaged across the four blocks for both the first and second field trials (Fig. 3). Factorial ANOVA failed to show a significant interaction between virus and brightener in either the first or second field trial. In subsequent analyses that excluded interaction terms, virus was the only factor explaining significant amounts of variation in feeding

**Table 3. Factorial analysis of variance showing the factors explaining significant amounts of variation in feeding damage under greenhouse conditions**

| Factor[a] | *F* ratio (df) | *P* value[b] |
|---|---|---|
| Replicate | 3.20 (3) | 0.0400 |
| Virus | 170.91 (1) | 0.0001 |
| Brightener | 4.79 (1) | 0.0380 |
| Formulation | 0.39 (1) | 0.5370 |

Damage data were $\log_{10}$ transformed and subjected to factorial ANOVA using the general linear model. Data satisfied assumptions of normality of error and homogeneity of variance. Analysis based on treatment means from four replicates.

[a] All possible interaction terms were included in the initial model, but none were significant. Results shown are from a subsequent analysis that did not contain interaction terms.

[b] Factors explaining significant amounts of variation in feeding damage indicated by *P*-values $< 0.05$.

damage in both the first ($F = 14.60$; df = 1, 13; $P = 0.002$) and second field trial ($F = 79.73$, df = 1, 13; $P = 0.0001$). Replicate, bait and brightener did not have significant effects on feeding damage. Laboratory bioassays of baits from both field trials resulted in 100% mortality of larvae fed baits containing AgipMNPV but no mortality in larvae fed non-AgipMNPV baits.

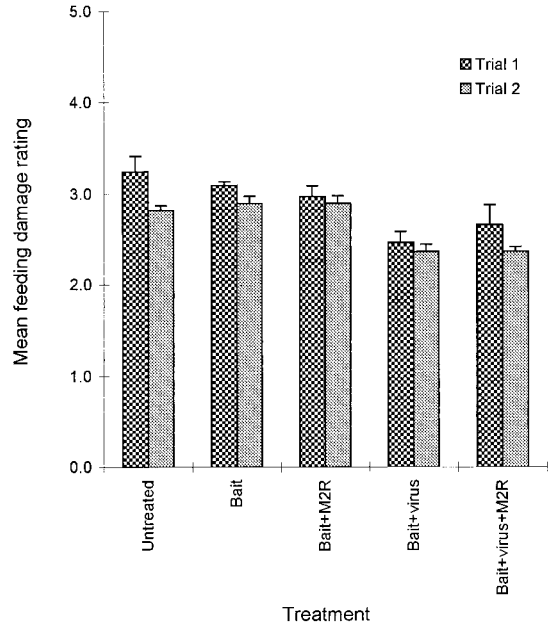


**Fig. 3.** Mean feeding damage ratings averaged across blocks for the five treatments used in the first and second field trials. Damage rating of 5 corresponds to cut seedlings, whereas 1 corresponds to undamaged seedlings (see *Materials and Methods*). Bars show standard errors.

## Discussion

Laboratory bioassays showed AgipMNPV to have high activity against *A. ipsilon* larvae. The $LD_{50}$ estimate for third-instar *A. ipsilon* fed AgipMNPV (330 OBs per larva) was much lower than published $LD_{50}$ estimates for second-instar *A. ipsilon* exposed to *Agrotis segetum* MNPV (44,000 OBs/larva) and *Autographa californica* MNPV (670,000 OBs/larva), but were similar to those for second-instar *A. ipsilon* exposed to *Heliothis armigera* NPV (105 OBs/larva) (Abol et al. 1989). The addition of 0.5% M2R to AgipMNPV treatments significantly reduced $LD_{50}$ estimates in third-instar *A. ipsilon* by ≈160-fold to ≈2 OBs per larva. This magnitude of viral enhancement by an optical brightener compares with 116–303,000-fold seen in *Spodoptera frugiperda* (J. E. Smith) (Hamm and Shapiro 1992; Shapiro and Hamm 1999), 42–1,670-fold seen in *L. dispar* (Shapiro and Robertson 1992; Farrar et al. 1995; Shapiro and Argauer 1995; Argauer and Shapiro 1997), 15–1,584-fold seen in *Pseudoplusia includens* (Walker) (Zou and Young 1996), 119-fold seen in *Spodoptera exigua* (Hübner) (Shapiro 2000), and 3.3-fold in *Choristoneura occidentalis* Freeman (Li and Otvos 1999).

In contrast to most previous studies, our results showed no direct effect of M2R on survival times. In previous studies, lethal times were determined for the same virus dose in the presence or absence of optical brightener, and any reductions in $LT_{50}$ estimates for the virus-brightener treatment were attributed to effects of brightener. However, because optical brighteners greatly reduce lethal doses, the virus-brightener treatments cause higher mortality than virus alone. Virus-brightener treatments thus constitute a greater relative viral dose than treatments consisting of the same dose of virus alone (despite the same absolute number of OBs), and since it is already known that increases in virus dose cause reductions in lethal time (Van Beek et al. 1988), the finding of lower $LT_{50}$ estimates for virus-brightener treatments would be expected. In studies designed in this way it is thus impossible to determine whether reductions in lethal times are due to brightener effects, dose effects, or a combination of both. An additional problem with some of these studies is that survivors were not excluded from the analysis of the lethal time data. If survivors are not excluded from the analysis, $LT_{50}$ estimates from different treatments should only be compared if both treatments cause similar levels of mortality in the test subjects (Farrar and Ridgway 1998). If $LT_{50}$ estimates are compared between treatments that cause significantly different levels of mortality, and survivors are not excluded from the analysis, $LT_{50}$ estimates are heavily inflated in treatments that cause lower levels of mortality. Our results show that when mortality levels are matched for virus versus virus-brightener treatments and survivors are excluded from the analysis, survival times are not significantly affected by brightener, although they are significantly affected by virus dose. We suggest this is also likely to be the case in other virus-brightener systems.

Greenhouse studies showed that AgipMNPV significantly reduced feeding damage to corn seedlings by *A. ipsilon* larvae. Although seedlings that received treatments containing AgipMNPV exhibited some leaf feeding damage, seedlings receiving nonvirus treatments were almost completely consumed. M2R

caused small but significant reductions in feeding damage which were independent of any effects attributable to virus, and could have resulted from the feeding deterrent effects of stilbene optical brighteners (Farrar et al. 1995, Farrar and Ridgway 1997, Vail et al. 1999). The interaction between virus and brightener was not significant, indicating that the presence of M2R in treatments did not lower feeding damage below that observed with virus alone. Thus, at the virus doses used in these greenhouse studies, there was no beneficial effect of M2R as a UV protectant or as a viral enhancer. The absence of any UV protectant benefits may be explained by the fact that greenhouse glass filters out much of the UV radiation in sunlight, whereas the absence of viral enhancing effects may be explained by the fact that even AgipMNPV treatments lacking M2R were more than potent enough to cause very high levels of mortality. Indeed all larvae in virus treated trays were dead 5 d after treatment.

In both replicated field trials, AgipMNPV significantly reduced feeding damage to corn seedlings by *A. ipsilon.* In contrast to the greenhouse studies, where seedlings in nonvirus treatments were almost completely destroyed, seedlings in nonvirus plots in the field merely exhibited higher levels of leaf feeding damage. There was little cutting damage to seedlings in the field trials. Chemical insecticides are usually applied against *A. ipsilon* populations in the field when 3% or more of corn seedlings are cut by fifth or earlier instars (Rice 1999). This level of damage was not observed in either of the AgipMNPV field trials. Evidently the lower seedling density in the field plots relative to the greenhouse trials, coupled with environmental mortality factors such as weather and predation by natural enemies, greatly reduced the feeding pressure on the corn seedlings, despite a larval infestation rate of twice that used in the greenhouse. Greater differences in feeding damage would have been apparent between AgipMNPV and nonvirus treatments had feeding pressure been higher in the field trials. Field trials by Johnson and Lewis (1982) demonstrated significant reductions in feeding damage to corn seedlings by *A. ipsilon* following applications of AcMNPV and *Rachiplusia ou* MNPV (Harrison and Bonning 1999) baits. These viruses were highly effective at reducing damage by first and second-instars, but were much less effective against third-instars, and laboratory bioassays of AcMNPV and RoMNPV baits caused only 55% mortality in third-instar *A. ipsilon* after 14 d (Johnson and Lewis 1982). In contrast, laboratory bioassays of the baits from the AgipMNPV field trials caused 100% mortality after only 8 d. Data from both sets of field trials failed to show significant interactions between AgipMNPV and M2R.

Despite the apparent promise shown by M2R as a viral enhancer in laboratory studies, there was no evidence that M2R acted as a UV protectant or viral enhancer under greenhouse or field conditions. Although previous studies have documented a beneficial effect of the inclusion of optical brighteners in baculovirus formulations, these studies tended to be in systems where the plant canopy offered significant protection from sunlight (Webb et al. 1994a, Webb et al. 1994b, Zou and Young 1996). In crop systems that are more open, such as cotton, the benefits of including optical brighteners in formulations appear to be more marginal, because the brightener itself is degraded by UV light within a few days (Vail et al. 1999). The high efficacy of AgipMNPV baits that did not contain M2R, may also have obscured potential beneficial effects of the brightener. The real utility of M2R may lie in obtaining similar levels of pest suppression at lower virus application rates, rather than from enhancement of viral activity at more conventional application rates of $10^{12}$ OBs/ha.

In summary, AgipMNPV is highly active against third-instar *A. ipsilon.* The optical brightener M2R significantly reduced the $LD_{50}$ by ≈160-fold, but had no direct effect on $ST_{50}$ estimates. AgipMNPV significantly reduced feeding damage to corn seedlings by *A. ipsilon* in greenhouse and field studies, although the addition of M2R produced no improvements in virus performance at the virus application rates used in this study. In an appropriately designed pest management program it seems likely that AgipMNPV could be used to suppress populations of early and middle-instar *A. ipsilon,* although slow speed of kill and high production costs continue to make it difficult for baculoviruses such as AgipMNPV to compete with chemical insecticides (Moscardi 1999). Nevertheless, the soaring cost of developing new chemical insecticides, coupled with increasing public concern about pesticide residues on food, may lead to greater interest in using baculoviruses for pest management in the future.

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